

Atty Dkt. No.: UCAL-234
USSN: 09/884,875

AMENDMENTS TO THE CLAIMS:

1. **(Previously Presented)** A method for identifying an agent that modulates NF- κ B activity in transcription of a gene in a eukaryotic cell, the method comprising:
contacting a candidate agent with a eukaryotic cell in vitro, wherein the eukaryotic cell comprises detectably labeled RelA; and
detecting a level of deacetylated RelA;
wherein detection of an increase in the level of deacetylated RelA in the presence of the candidate agent compared to a level of deacetylated RelA in the absence of the candidate agent indicates that the agent inhibits activity of NF- κ B in gene transcription in the eukaryotic cell.
2. **(Original)** The method of claim 1, wherein RelA is detectably labeled so that deacetylation results in release of the detectable label from RelA, and said detecting of deacetylated RelA is by detecting a decrease in detectably labeled RelA.
3. **(Original)** The method of claim 1, wherein RelA is detectably labeled so that deacetylation results in release of the detectable label from RelA, and said detecting of deacetylated RelA is by detecting released detectable label.
4. **(Previously Presented)** The method of claim 1, wherein said detecting is performed in the presence of histone deacetylase 3 (HDAC3).
5. **(Previously Presented)** The method of claim 1, wherein detection of deacetylated RelA is by detection of export of RelA from the nucleus of the cell, wherein detection of RelA export indicates RelA is deacetylated.
6. **(Previously Presented)** The method of claim 1, wherein detection of deacetylated RelA is by detection of an increase in RelA binding to I κ B α .

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7. **(Currently Amended)** A method for identifying a substance that inhibits NF- κ B activity, comprising testing a substance for activity in deacetylation of RelA or inhibition of RelA acetylation, the method comprising the steps of:

exposing a sample comprising a detectably labeled RelA to a test substance;

comparing the level of deacetylated RelA in the sample comprising the test substance to the level of deacetylated RelA in a sample without the test substance; and

determining whether the level of deacetylated RelA is greater in the sample exposed to the test substance than a level of deacetylated RelA in the sample without the test substance;

wherein an increase in deacetylated RelA in the presence of the test substance indicates the test substance inhibits NF- κ B activity.

8. **(Original)** The method according to claim 7, wherein the exposing step includes using an extract of cells, which were treated with an inducer for NF- κ B activation, or a fraction of said extract.

9. **(Original)** The method according to claim 7, wherein a cell-free system is used for the exposing step.

10. **(Original)** The method according to claim 9, wherein RelA is bound to a support.

11-18 (Cancelled)

19. **(Previously Presented)** The method of claim 1, wherein said contacting is in the presence of a protein or protein complex that acetylates RelA.

20. **(Previously Presented)** The method of claim 19, wherein the protein that acetylates RelA is CBP or p300.

21. **(Previously Presented)** The method of claim 1, wherein RelA is within a eukaryotic cell, which cell contains CBP and p300.

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22. **(Previously Presented)** The method of claim 1, wherein said contacting is in the presence of HDAC3 and wherein detection of an increase of deacetylated RelA in the presence of the candidate agent is compared to a level of deacetylated RelA in the absence of the candidate agent.

23. **(Previously Presented)** The method of claim 8, wherein the extract comprises p300 and CBP.

24. **(Previously Presented)** The method of claim 23, wherein the extract comprises HDAC3.

25. **(Currently Amended)** A method for identifying an agent that modulates NF- κ B activity in transcription of a gene in a eukaryotic cell, the method comprising:

contacting a candidate agent with a eukaryotic cell in vitro, wherein the eukaryotic cell comprises a recombinant nucleic acid comprising a nucleotide sequence encoding RelA operably linked to a detectable label; and

detecting the level of deacetylated RelA;

wherein detection of an increase in deacetylated RelA in the cytoplasm in the presence of the candidate agent compared to a level of deacetylated RelA in the cytoplasm in the absence of the candidate agent indicates that the agent inhibits activity of NF- κ B in gene transcription in the eukaryotic cell.

26. **(Previously Presented)** The method of claim 25, wherein said detecting is performed in the presence of histone deacetylase 3 (HDAC3).

27. **(Canceled)**

28. **(Previously Presented)** The method of claim 25, wherein said detectable label is a fluorescent polypeptide.

29. **(Previously Presented)** The method of claim 25, wherein said contacting is in the presence of a protein or protein complex that acetylates RelA.

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30. **(Previously Presented)** The method of claim 29, whercin the protein that acetylates RelA is CBP or p300.

31. **(Previously Presented)** The method of claim 25, wherein the eukaryotic cell comprises CBP and p300.

32.-42. **(Canceled)**

43. **(Previously Presented)** A method for identifying an agent that modulates NF- κ B activity in transcription of a gene in a eukaryotic cell, the method comprising:
contacting a candidate agent with a eukaryotic cell in vitro,
immunoprecipitating a cell lysate of the eukaryotic cell to immunoprecipitate RelA; and
contacting the immunoprecipitated RelA with an anti-acetylated lysine antibody that binds acetylated RelA to detect a level of acetylated RelA,

whercin detection of a decrease in the level of acetylated RelA in the presence of the candidate agent compared to a level of acetylated RelA in the absence of the candidate agent indicates that the agent inhibits activity of NF- κ B in gene transcription in the eukaryotic cell.

44. **(Previously Presented)** The method of claim 43, wherein the eukaryotic cell comprises a recombinant nucleic acid comprising a nucleotide sequence encoding T7-RelA, and wherein the immunoprecipitating is done using an anti-T7 antibody.

45. **(Previously Presented)** The method of claim 43, wherein said detecting is performed in the presence of histone deacetylase 3 (HDAC3).

46. **(Previously Presented)** The method of claim 43, whercin said contacting is in the presence of a protein or protein complex that acetylates RelA.

47. **(Previously Presented)** The method of claim 46, whercin the protein that acetylates RelA is CBP or p300.

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48. (Previously Presented) The method of claim 43, wherein the eukaryotic cell comprises CBP and p300.